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# Separation and quantification of ropinirole and some impurities using capillary liquid chromatography

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**Abstract**

Ropinirole, 4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one, is a potent anti-Parkinson's disease drug developed by SmithKline Beecham Pharmaceuticals. Capillary liquid chromatography (CLC) was used for the separation and quantification of ropinirole and its five related impurities, potentially formed during its synthesis. A simultaneous optimization of three mobile phase parameters, i.e., pH, buffer concentration and acetonitrile content was performed employing an experimental design approach which proved a powerful tool in method development. The retention factors of the investigated substances in different mobile phases were determined. Baseline resolution of the six substances on a $C_{18}$ reversed stationary phase was attained using a mobile phase with an optimized composition [acetonitrile–8.7 m*M* 2-(*N*-morpholino)ethanesulfonic acid adjusted to pH 6.0 (55:45, v/v)]. It was shown that CLC, operated in the isocratic mode under the mobile phase flow-rate of 4 μl/min, can determine the level of these impurities, down to a level of 0.06% of the main component within 25 min.


*Keywords:* Ropinirole

## 1. Introduction

The earliest attempts using packed narrow-bore columns in high-performance liquid chromatography (HPLC) were made at the end of the 1960s by Horvath et al. [1,2], who carried out the first capillary liquid chromatography (CLC) experiments in packed stainless steel capillaries of 1 mm inner diameter. Nowadays, CLC employing shorter and narrower columns packed with stationary phases of the particle diameters of 5 μm, 3 μm or 1 μm is becoming a powerful and widespread separation tool in analytical chemistry [3–16].

The development of capillary HPLC is discussed in a detailed review on microcolumn LC [17]. Compared to conventionally sized HPLC, CLC exhibits certain benefits, such as higher performance, considerably lower requirements for mobile phase components and sample amounts and an improvement in detection sensitivity. Although the cost of CLC equipment is comparable with that of HPLC, the running expenses of the CLC equipment are considerably lower than those of conventional HPLC. The reduced consumption of organic modi-

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fiers in CLC is also of benefit to the environment. CLC is a suitable technique for solving pharmaceutical problems, such as main component assays, impurity profiles and the determination of metabolites in body fluids. The sample amounts available in some these applications may be very low; however, to secure a sufficient reliability of the analytical results, each analysis may be repeated several times.

Ropinirole, 4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one, is a potent anti-Parkinson's disease drug which has been developed by SmithKline Beecham Pharmaceuticals. This compound of interest may be accompanied by five structurally related impurities originating from the synthesis procedure. In another paper [18], capillary zone electrophoresis (CZE) has already been used for dissociation constants determination, separation and quantification of ropinirole and these five related impurities.

This article is focused on the application of CLC to separation of a set of six highly similar compounds, one of which is a newly developed pharmaceutical drug. The separation has been optimized using an experimental design approach which has been shown as a powerful tool in method development. Retention factors of the investigated compounds have been determined in different mobile phases on a $C_{18}$ stationary phase. The technique was further used for quantitative determination of ropinirole and its most common impurities.

## 2. Experimental

### 2.1. Chemicals

The mobile phase compositions corresponding to individual points of the experimental design are given in Table 1. Acetic acid, sodium acetate, sodium hydroxide, 2-(*N*-morpholino)ethanesulfonic acid (MES), 3-(*N*-morpholino)propanesulfonic acid (MOPS) and acetonitrile were purchased from Merck (Darmstadt, Germany). Acetate buffer was used for the preparation of the mobile phases of pH 5 and 4.8. MES was employed to prepare the mobile phases of pH 6 and MOPS was used for the preparation of the mobile phases of pH 7 and 7.2. The buffers were adjusted to a certain pH-value in purely aqueous solutions, then a required amount of acetonitrile was added to the solution and, after heating, the solution was diluted with water to the final concentration.

Thiourea (Sigma, St. Louis, MO, USA) in a 1 m*M* concentration was employed as the dead time marker. Ropinirole and its five common impurities (for structures and numerical labels see Fig. 1) were

Table 1
Mobile phase compositions used in the experimental design

| Mobile phase No. | pH | Buffer concentration (m*M*) | Acetonitrile content (%) |
|---|---|---|---|
| 1 | 5.0 | 2.0 | 40 |
| 2 | 5.0 | 2.0 | 70 |
| 3 | 5.0 | 8.0 | 40 |
| 4 | 5.0 | 8.0 | 70 |
| 5 | 7.0 | 2.0 | 40 |
| 6 | 7.0 | 2.0 | 70 |
| 7 | 7.0 | 8.0 | 40 |
| 8 | 7.0 | 8.0 | 70 |
| 9 | 6.0 | 5.0 | 55 |
| 10 | 4.8 | 5.0 | 55 |
| 11 | 7.2 | 5.0 | 55 |
| 12 | 6.0 | 1.4 | 55 |
| 13 | 6.0 | 8.7 | 55 |
| 14 | 6.0 | 5.0 | 37 |
| 15 | 6.0 | 5.0 | 73 |

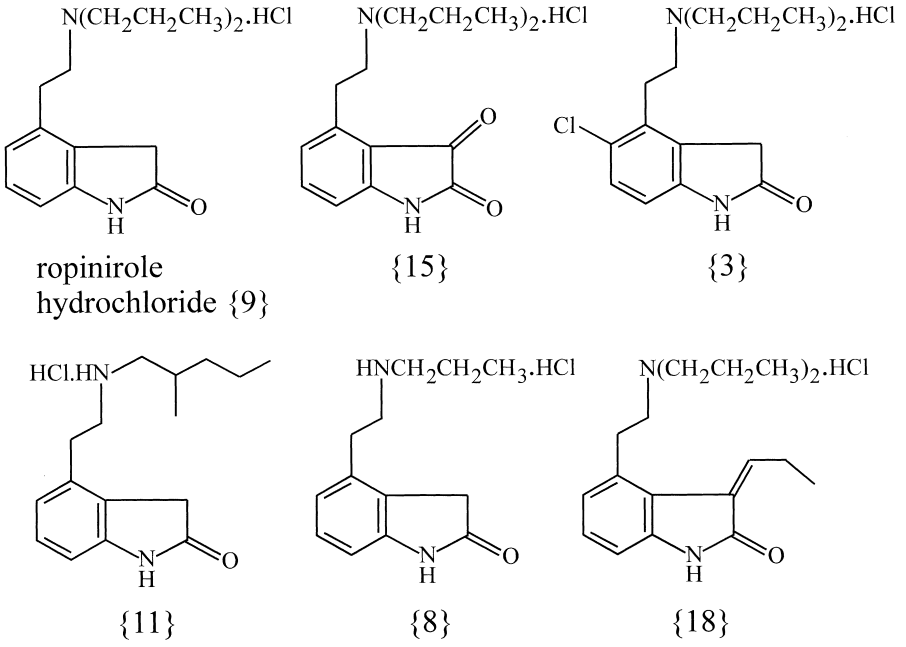


Fig. 1. Structures and numerical labels of ropinirole and its impurities.

provided by SmithKline Beecham Pharmaceuticals (Tonbridge, UK). Nucleosil 100-5 $C_{18}$, 5 μm (Macherey-Nagel, Düren, Germany) was used as the stationary phase in packed capillary columns. The water used for preparation of all the solutions was purified with a Milli-Q water purification system (Millipore, USA). All the chemicals were of the analytical-reagent grade purity and were used as received.

### *2.2. Apparatus*

A Syringe pump Phoenix 20 CU (Carlo Erba Instruments, Milan, Italy), a 60-nl injection valve C14W (VICI-AG Valco Europe, Schenkon, Switzerland) and a programmable absorbance detector, Model 785A (Applied Biosystems, Ramsey, USA) operated at a constant wavelength of 250 or 254 nm were used in all of the CLC experiments. All the quantification experiments were performed at a wavelength of 254 nm. Fused silica capillary columns (Chrompack, Middelburg, The Netherlands) packed with Nucleosil 100-5 $C_{18}$, 5 μm (Macherey-Nagel) had a packed part of 41 cm×320 μm I.D. and an open part of 38 cm×75 μm I.D. All the packed capillary columns were prepared using the packing procedure published elsewhere [19,20]. The sample amounts loaded in the separation experiments, employing the 60-nl injection valve and injecting about 0.5 m*M* analyte solutions, were 9 ng (i.e., 30 pmol). The mobile phase flow-rate of 4 μl/min was used in all the CLC experiments.

## 3. Results and discussion

### *3.1. Separation*

Ropinirole and its impurities show different affinities for chromatographic stationary and mobile phases due to differences in their molecular structure. The resultant differences in the distribution constants may be used for separation of the investigated substances by CLC. Many mobile phases can be applied to the separation on a given stationary phase. However, only a few mobile phases permit sufficient specificity, i.e., baseline resolution, for the investigated compounds on a certain stationary

phase. To find a proper composition of the mobile phase for separation of a given set of analytes on a certain stationary phase, an experimental design approach may be utilized, as this method makes it possible to optimize many mobile phase parameters at the same time [21]. When an experimental design approach is used, these mobile phase parameters are optimized simultaneously, considering their mutual interactions [22]. This method is capable of locating an optimum for the mobile phase composition within the investigated intervals of the mobile phase parameters to be optimized.

Three mobile phase parameters, i.e., pH, buffer concentration and acetonitrile content, were chosen to influence the selectivity of the CLC separation on a $C_{18}$ reverse stationary phase. As these three parameters may mutually interact, a central composite experimental design [23] (schematically outlined in Fig. 2) was applied to optimize the parameters simultaneously and to find an optimum of the mobile phase composition within the investigated three-dimensional space of the parameters.

The mobile phase compositions corresponding to the individual points of the experimental design are given in Table 1. A mixture of the test compounds was separated step-by-step on a capillary column packed with a $C_{18}$ reverse stationary phase using 15 different mobile phases which were selected by the experimental design. Chromatograms of all the separations were recorded, individual peaks in all chromatograms were identified and retention times obtained from the chromatograms were utilized to calculate the retention factors of the investigated compounds in each mobile phase. These data are given in Table 2 where the values are the arithmetic means of at least two experimental values.

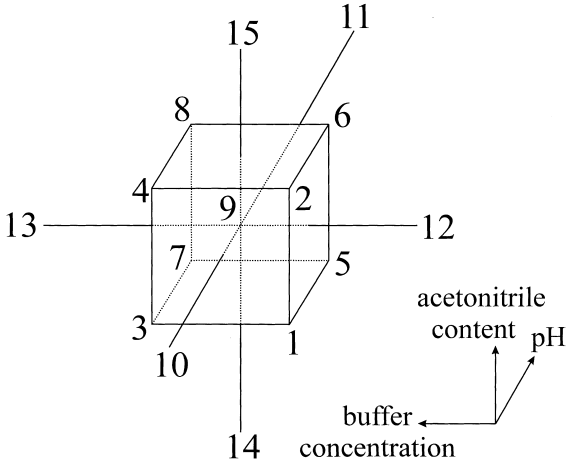


Fig. 2. A central composite experimental design in the three-dimensional space of the test parameters.

From all the test mobile phases, mobile phases 10 and 13 with a column packed with the specific $C_{18}$ reverse stationary phase leads to a baseline separation of all the six studied compounds as apparent from Fig. 3. The shorter analysis time (i.e., 25 min) was attained with mobile phase 13. In the case of mobile phase 10, the analysis time was longer (i.e., 27 min). All the other investigated mobile phases yielded a worse resolution of the analytes. All the studied mobile phases exhibited the same elution order of the analytes (i.e., {8}, {9}, {15}, {11}, {3} and {18}) with an exception for mobile phases 4 and 8, with which the elution order of the compounds {11} and {3} was reversed.

### *3.2. Quantification*

Mobile phase 13, containing acetonitrile–8.7 m*M* MES adjusted to pH 6.0 with sodium hydroxide (55:45, v/v), was chosen to perform the quantification experiments, as this mobile phase guaranteed a baseline resolution of the analytes and a relatively short analysis time. To confirm linearity of the detector response to the concentration of the test compounds, calibration curves (peak area versus concentration expressed in μg/ml) were measured with ropinirole in a low concentration range from 0 to 25 μg/ml and a high concentration range from 0 to 2500 μg/ml and with the ropinirole impurities in the low concentration range from 0 to 25 μg/ml. The experimental data were subjected to linear regression obtaining the results that are summarized in Table 3.

All the intercepts of the calibration curves in Table 3 were not found to be significantly different from zero at a significance level of $\alpha=0.05$ using the *t*-test for intercepts where $sd_a$ is the standard deviation of $a$ ($t=a/sd_a$). A good agreement between the experimental points and the linear calibration curves was found as follows from the correlation coefficients. In the worst case the correlation coefficient

Table 2
Retention factors of the investigated compounds in 15 different mobile phases

| Mobile phase | Compounds[a] | | | | | |
|---|---|---|---|---|---|---|
| | {8} | {9} | {15} | {11} | {3} | {18} |
| 1 | 1.48 | 2.81 | 3.12 | 4.29 | 4.46 | 7.69 |
| 2 | 1.54 | 2.15 | 2.29 | 2.44 | 2.66 | 3.60 |
| 3 | 0.62 | 1.38 | 1.56 | 2.21 | 2.38 | 4.42 |
| 4 | 0.46 | 0.74 | 0.80 | 1.44 | 1.00 | 3.38 |
| 5 | 5.58 | 11.58 | 12.48 | 17.09 | 18.04 | 32.92 |
| 6 | 2.94 | 3.96 | 4.22 | 4.52 | 4.77 | 6.36 |
| 7 | 1.82 | 3.92 | 4.42 | 6.06 | 6.89 | 13.26 |
| 8 | 0.84 | 1.15 | 1.23 | 1.94 | 1.45 | 2.52 |
| 9 | 1.06 | 1.70 | 1.91 | 2.17 | 2.31 | 3.45 |
| 10 | 0.72 | 1.30 | 1.46 | 1.68 | 1.84 | 2.90 |
| 11 | 1.67 | 2.79 | 3.07 | 3.50 | 3.94 | 6.30 |
| 12 | 4.24 | 6.70 | 7.48 | 8.51 | 8.96 | 13.40 |
| 13 | 0.85 | 1.49 | 1.68 | 1.92 | 2.10 | 3.28 |
| 14 | 1.76 | 3.76 | 4.18 | 6.28 | 6.62 | 12.44 |
| 15 | 0.91 | 1.23 | 1.31 | 1.37 | 1.50 | 1.93 |

[a] For structures see Fig. 1.

value (i.e., $r=0.995$) observed for compound {9}, the experimental point variation was explained from 99.0% (i.e., $100 \cdot r^2$) by the calibration curve variation. The limits of detection were determined as three-times root mean squared baseline noise [21]. For all the test substances, the linearity of the calibration curves obtained in the CLC experiments was comparable with that found in the CZE experiments [18]. On the other hand, the limits of detection (LODs) of the test compounds observed in the CLC were higher than those in the CZE [18] showing a lower signal-to-noise ratio of CLC for all the investigated impurities in comparison with CZE under the given experimental conditions. The utilization of the more-light-absorbing MES and the higher content of acetonitrile in the CLC mobile phase 13 compared to the less-light-absorbing borate and magnesium sulfate and the lower content of acetonitrile in the CZE running buffer [18] could be one of the reasons for differences in the LODs. A lower separation performance leading to lower and broader peaks in the CLC experiments in comparison with the CZE experiments [18] could also contribute to the LOD differences.

Mobile phase 13 was also used to analyze the impurity profile of two real samples of ropinirole from its synthetic impurities; Fig. 4. As the slopes of the calibration curves, representing the CLC sensitivity for the individual analytes, were comparable and the level of impurities was very low, an internal normalization evaluation method was used to quantify the percentage of impurities in the ropinirole batches. The mean values of the area percent (i.e., the percent impurity quantity), the relative standard deviations and the impurity concentrations in the injected solution are summarized in Table 4. The highest impurity level determined, was 1.16% with relative standard deviation of 1.5%. Employing conventional HPLC, the following impurity concentrations were determined in the first ropinirole batch using the internal normalization evaluation method at the wavelength of 254 nm [24]: 0.1% of {8}, 0.2% of {11}, 1.1% of {15} and 0.3% of {18}; {3} was not detected. There was thus a very good agreement between the CLC and HPLC data and a satisfactory agreement between the CLC and CZE data [18]. The small differences between the CLC and CZE may be due to the use of the internal normalization evaluation methods, which is very often applied in impurity profiles of pharmaceutical compounds, and due to the dependence of the peak area on the zone migration velocity in CZE. The impurity concentration in the injected solution calculated from the mean of area percent were higher than or comparable to the LODs determined as three-times root mean squared baseline noise with an

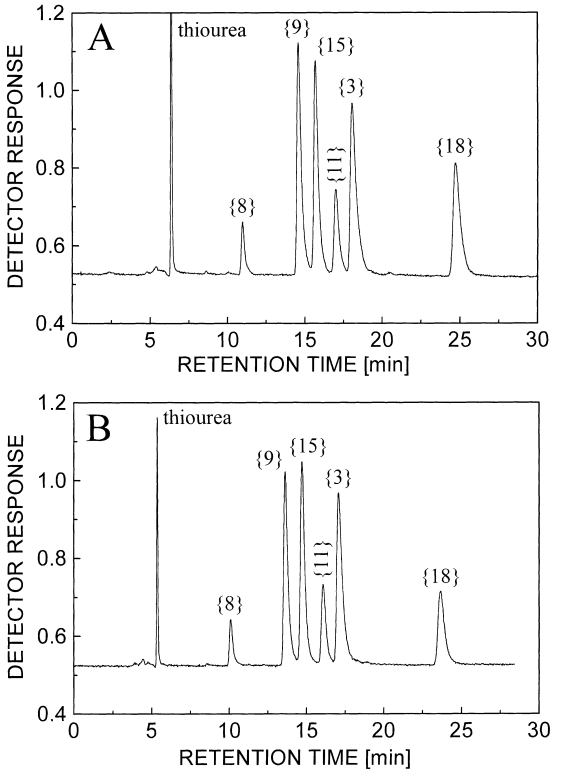


Fig. 3. Chromatograms of the analytes in mobile phase 10 containing acetonitrile–5 m*M* $CH_3COONa$ adjusted to pH 4.8 by $CH_3COOH$ (55:45, v/v) (A) and in mobile phase 13 consisting of acetonitrile–8.7 m*M* MES adjusted to pH 6.0 by NaOH (55:45, v/v) (B). Aqueous sample, ca. 0.15 mg/ml of each compound; injection volume, 60 nl; stationary phase, Nucleosil 100-5 $C_{18}$, 5 μm particle diameter; separation column, 41 cm×320 μm; mobile phase flow-rate, 4 μl/min; detection, 250 nm.

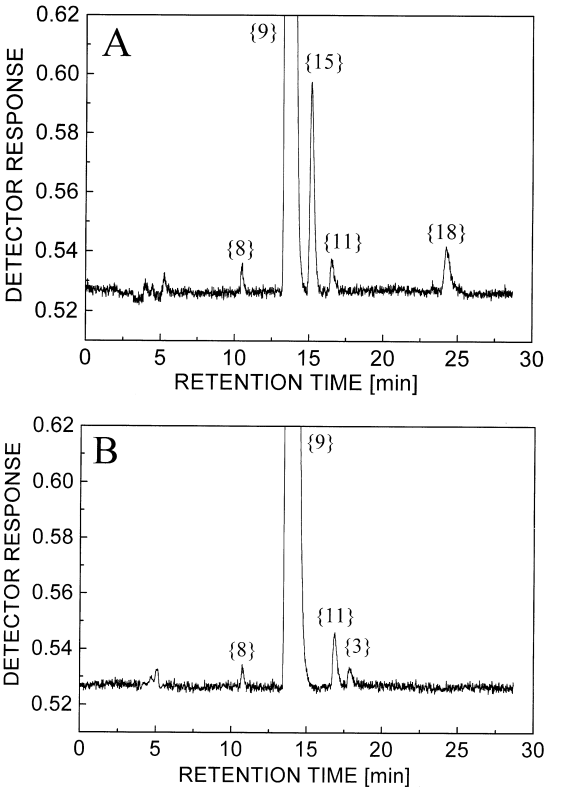


Fig. 4. Chromatograms of the No. 1 (A) and No. 2 (B) test batches of ropinirole in mobile phase 13 consisting of acetonitrile–8.7 m*M* MES adjusted to pH 6.0 by NaOH (55:45, v/v). Aqueous sample, 2 mg/ml; injection volume, 60 nl; stationary phase, Nucleosil 100-5 $C_{18}$, 5 μm particle diameter; separation column, 41 cm×320 μm; mobile phase flow-rate, 4 μl/min; detection, 254 nm.

Table 3
Linear regression parameters of the calibration curves [peak area=$a+b\cdot$conc. (μg/ml)] for ropinirole and its impurities, with the standard deviations in parentheses[a]

| Compound[b] | *a* (SD) | *b* (SD) | *r* | LOD (μg/ml) |
|---|---|---|---|---|
| {8} | 78 (215) | 498 (13) | 0.998 | 1.1 |
| {9} | 309 (421) | 600 (25) | 0.997[c] | 1.7 |
| | −7517 (44 871) | 564 (27) | 0.995[d] | |
| {15} | −263 (295) | 681 (18) | 0.998 | 1.3 |
| {11} | −287 (217) | 566 (13) | 0.999 | 2.1 |
| {3} | −48 (540) | 694 (32) | 0.996 | 1.9 |
| {18} | 297 (375) | 606 (23) | 0.997 | 2.4 |

[a] *a* is the intercept; *b* the slope; *r* the correlation coefficient and LOD the limit of detection.
[b] For structures see Fig. 1.
[c] For the low concentration range from 0 to 25 μg/ml.
[d] For the high concentration range from 0 to 2500 μg/ml.

Table 4
The impurity profile of the real sample Nos. 1 and 2 of ropinirole using the internal normalization evaluation method

| Compound[a] | Mean area (%)[b] | | RSD (%) | | Concentration[c] (μg/ml) | |
|---|---|---|---|---|---|---|
| | No. 1 | No. 2 | No. 1 | No. 2 | No. 1 | No. 2 |
| {8} | 0.09 | 0.06 | 2.7 | 2.9 | 1.8 | 1.2 |
| {9} | 98.24 | 99.59 | 0.04 | 0.07 | 2000 | 2000 |
| {15} | 1.16 | nd[d] | 1.5 | nd | 23.2 | nd |
| {11} | 0.18 | 0.27 | 2.6 | 0.8 | 3.6 | 5.4 |
| {3} | nd | 0.08 | nd | 1.9 | nd | 1.6 |
| {18} | 0.33 | nd | 2.8 | nd | 6.6 | nd |

[a] For structures see Fig. 1.
[b] Six repeated runs, $n=6$, for sample No. 1; six repeated runs, $n=6$, for sample No. 2.
[c] The analyte concentration in the injected solution.
[d] nd=Not detected.

exception for compound {3} in the second ropinirole batch.

## 4. Conclusions

Reversed-phase CLC has been demonstrated as a powerful separation method for structurally similar compounds. However, an optimization of the mobile phase composition was necessary to obtain a baseline resolution of all the substances. An experimental design approach was shown as the method enabling the simultaneous optimization of three various mobile phase parameters including their mutual interactions. This optimization technique proved as a powerful tool in the method development since it led to mobile phases providing a baseline resolution of ropinirole and its impurities on the $C_{18}$ reversed stationary phase. The work presented here underlines the importance of application of CLC separations of pharmaceutical compounds. CLC operated in the isocratic mode provides a rapid impurity profile method for ropinirole batches with an analysis time not exceeding 25 min. The reduced mobile phase flow-rate of 4 μl/min guarantees a considerable saving in preparation time, organic modifiers and use of mobile phases. It has been shown that impurity levels lower than 0.06% can be determined using CLC within a relatively short analysis time, having relative standard deviations below 3%. A very good agreement has been found between the impurity profiles determined by CLC and HPLC. Under the experimental conditions used in this study, the CLC method exhibited a lower signal-to-noise ratio for the ropinirole impurities but a higher peak area reproducibility in comparison with the CZE technique.

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